Available online at www.sciencedirect.com
SCIENCE DIRECT
Experimental Parasitology 112 (2006) 21–30
Experimental Parasitology
www.elsevier.com/locate/yexpr

Leishmania donovani: Differential activities of classical topoisomerase inhibitors and antileishmanials against parasite and host cells at the level of DNA topoisomerase I and in cytotoxicity assays

Valerie Jean-Moreno [a], Ricardo Rojas [a], Diego Goyeneche [a], Graham H. Coombs [b], John Walker [a,*]

[a] *Centro Internacional de Entrenamiento e Investigaciones Médicas (CIDEIM), Biochemistry and Molecular Biology Unit, Avenida 1 Norte No. 3-03, AA 5390, Cali, Valle de Cauca, Colombia*
[b] *Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, G12 8QQ, UK*

Received 8 June 2005; received in revised form 30 August 2005; accepted 31 August 2005
Available online 15 November 2005

Abstract

Different classes of topoisomerase (TOP) inhibitors and antitrypanosomatid agents exhibited variable efficacies against *Leishmania donovani* parasites and human mononuclear cells both at the level of DNA topoisomerase I (TOPI) catalytic activity and in cytotoxicity assays. Bis-benzimidazoles and the diamidine diminazene aceturate exhibited uniformly high efficacies against parasite and host enzymes as well as against parasite and mononuclear cells, but pentamidine showed around 2 orders of magnitude greater specificity for *Leishmania* TOPI and amastigote cells ($P < 0.05$). The protoberberine coralyne and the flavonoid quercetin were highly potent, but non-selective, inhibitors in vitro, although the latter showed slight selectivity for parasite TOPI. Camptothecin was selective for mononuclear cells at both levels ($P < 0.05$) and sodium stibogluconate was selective only at the enzyme level displaying 30-fold greater potency against parasite TOPI ($P < 0.05$). These data suggest that at least part of pentamidines' leishmanicidal activity may be mediated through TOPI inhibition, and support the feasibility of exploiting differences between *Leishmania* and human TOPs to develop modified compounds with improved selectivity.

Index Descriptors and Abbreviations: *Leishmania donovani*; DNA topoisomerase I; Enzyme inhibition; Cytotoxicity; Bis-benzimidazole; Camptothecin; Protoberberine; Flavonoid; Antileishmanials

1. Introduction

The protozoan parasite *Leishmania* spp. is the causative agent of a clinically diverse disease known as leishmaniasis that affects an estimated 12 million people (Desjeux, 2001; Herwaldt, 1999) and which is currently listed by the World Health Organization as the second most important protozoan disease after malaria in terms of patient mortalities (WHO Fact Sheet No. 116, 2000; WHO Report, 1999 [http://www.who.org]).

Treatment of leishmaniasis depends entirely upon chemotherapy and yet the available armoury of effective drugs is limited. Pentavalent antimonials, for many years the first-line of antileishmanial defence, have been rendered obsolete in several endemic regions (e.g., India) by the emergence of antimony-resistant parasites (Farault-Gambarelli et al., 1997; Sundar, 2001). The alkyl phospholipid compound miltefosine is established as an effective new oral therapy for Old World visceral leishmaniasis (Jha et al., 1999), but variable results were obtained in trials against parasites of the *L. Viannia* subgenus (Soto et al., 2004). As a consequence, treatment options for New World (muco)cutaneous leishmaniases remain restricted by the lack of novel

* Corresponding author. Fax: +157 2 667 2989.
E-mail address: john_walker@cideim.org.co (J. Walker).

0014-4894/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2005.08.014

agents combined with the limitations of existing second-line drugs such as pentamidine and amphotericin B (Herwaldt, 1999). Thus, there is an increasingly urgent need to define biochemical targets for rational development of antileishmanial drugs and treatment strategies.

In recent years, attention has focused upon the DNA topoisomerases (TOPs) of trypanosomatid parasites (*Leishmania* spp. and *Trypanosoma* spp.) as potential targets for drug development. These enzymes play pivotal roles in modulating DNA topology during replication, transcription, recombination, and repair, and are established sites of action for clinical antibacterial and antitumour agents (Hooper, 1998; Liu, 1989). TOPs operate by a three-step catalytic mechanism involving cleavage, strand-passage, and religation of DNA strands, and are classified as Type 1 or Type 2 enzymes according to their specific modes of action upon one or both strands of the DNA helix (Wang, 1985). TOPs I and II of trypanosomatids exhibit significant structural and biochemical variations from the corresponding human enzymes, and have important functions in organizing the kinetoplast DNA (kDNA) network unique to these parasites (Burri et al., 1996; Cheesman, 2000; Das et al., 2001; Villa et al., 2003). Furthermore, increasing evidence for differential interactions of parasite and human TOPs with classical TOP inhibitors including poisons and DNA minor groove-binding ligands (MGBLs) has raised hopes of designing trypanosomatid-specific inhibitors. TOP poisons act by stabilizing the enzyme–substrate intermediate ("cleavable-complex") thus inducing TOP-mediated DNA cleavage, replication arrest and apoptosis. MGBLs bind to the DNA minor groove thereby interfering with substrate binding and catalysis, and apart from a few notable exceptions (e.g., bis-benzimidazoles), most do not promote DNA cleavage (Burri et al., 1996; Cheesman, 2000; Pommier et al., 1998). Camptothecin and the bis-benzimidazoles Hoechst-33342 and -33258 were shown to target trypanosomatid TOPs causing irreversible DNA damage and cell death, but the relative sensitivities of parasites and host mononuclear cells varied considerably at the enzyme and/or cellular levels (Bodley and Shapiro, 1995; Marquis et al., 2003a; Shapiro et al., 1989; Walker and Saravia, 2004). Similarly, specific representatives of the protoberberine class of TOPI poisons (Marquis et al., 2003b), and novel bis-napthoquinone and flavonoid derivatives have been reported as *Leishmania*-selective agents (Mittra et al., 2000; Ray et al., 1998). Trypanosomatid TOPs have also been suggested (with varying degrees of experimental support) as targets for current clinical antitrypanosomal and antileishmanial drugs, including diminazene aceturate (Berenil), pentamidine, and pentavalent antimonials (Basselin et al., 1998; Chakraborty and Majumder, 1988; Fox et al., 1990; Shapiro and Englund, 1990; Walker and Saravia, 2004).

In this study, we have examined the relative inhibitory effects of model TOP poisons (camptothecins, protoberberines, flavonoids, and fluoroquinolones), MGBLs (bis-benzimidazoles and diamidines), and antileishmanials (sodium stibogluconate and Amphotericin B) upon the catalytic activities of *Leishmania donovani* and human monocyte TOPI, in parallel with in vitro cytotoxicity analyses of both parasite life stages and human mononuclear cells. Our findings support the feasibility of specifically targeting *Leishmania* TOPs through rational drug design and contribute to the understanding of the modes of action of current antileishmanials.

2. Materials and methods

2.1. Inhibitors

Additive-free preparations of the pentavalent antimonial drug sodium stibogluconate (SSG) (lot no. BL06916) were obtained as a powdered formulation from the Walter Reed Army Institute of Research. SSG concentrations were calculated in terms of μM of pentavalent antimony (Sb^V) using the following molecular weight (M_r) data: Sb^V, M_r 122; SSG, M_r 746. All other inhibitors were purchased from Sigma (St. Louis, Missouri). SSG, Hoechst compounds, and diminazene aceturate were prepared in ultra-pure water and the remaining compounds were solubilized in dimethylsulphoxide (DMSO). Stock drug solutions were sterilized by filtration and either used immediately or stored at –70 °C. For ED_{50} assays, serial drug dilutions were made in phosphate-buffered saline (PBS).

2.2. Biological material

2.2.1. Human U-937 monocytes and macrophages

The human promonocytic cell line U-937 (ATCC CRL-159302) was cultured at 37 °C in a 5% CO_2 atmosphere, using RPMI 1640 medium containing 1% glutamine, 10% foetal calf serum, 100 IU penicillin, and 100 μg/ml streptomycin (Sundstrom and Nilsson, 1976). Monocytes were harvested during the logarithmic stage of growth (48 h after subculture), quantified by microscopy and either stored in liquid nitrogen for subsequent preparation of enzyme extracts or transformed to macrophages for cytotoxicity testing. Monocytes were transferred to standard medium containing 100 ng/ml phorbol myristate acetate (PMA; Sigma) for the induction of adherence and differentiation into macrophages (Bosque et al., 1998), and samples containing 1×10^5 cells (for infection with parasites and drug testing) were seeded into 96-well microtitre plates and incubated for 96 h (at 37 °C, 5% CO_2) (Bosque et al., 1998), prior to infection with parasites and/or drug testing (see below).

2.2.2. Leishmania parasites

Promastigotes of *L. donovani* (WHO reference strain MHOM/IN/80/DD8) were cultured at 25 °C by standard procedures in Schneider's Drosophila medium containing 10% heat-inactivated foetal bovine serum (Gibco, Grand Island, New York), 1% glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin (Bosque et al., 1998). For enzyme studies, logarithmic phase parasites (at a mean cell density of 0.5×10^6 promastigotes per ml) were collected at 72 h